The CiPA Profile of Two Adenosine Uptake Inhibitors, dilazep and dipyridamole

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INTRODUCTION

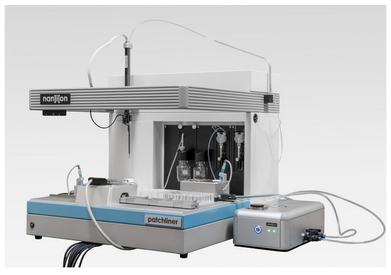
- Inhibition of adenosine uptake is a common mechanism of action for vasodilators and anti-platelet medications (ref. 1). Two examples, dipyridamole and dilazep, have been marketed for years but have not been tested using the CiPA paradigm.
- The objective of this work was to test dipyridamole and dilazep against seven cardiac ion channels, use this data to predict *in silico* their proarrhythmic potential, and confirm these data in human induced pluripotent stem cell cardiomyocytes (hIPSC-CMs), as per the CiPA paradigm (ref. 2).

MATERIALS AND METHODS

- (1) The activity of two compounds, dilazep and dipyridamole (Sigma, UK) was tested against 7 cardiac ion channels (the "CiPA ion channel panel"; hERG, hNaV1.5 peak and late current, hCaV1.2, hKir2.1, hKvLQT1 and Kv4.3) stably expressed in recombinant cell lines. Ion currents were measured by automated patch-clamp at ambient temperature (PatchLiner, Nanion Technologies).
- (2) The resulting IC_{50}, % inhibition and Hill Coefficients were used as inputs for the in-silico Action Potential (isAP) model to simulate the impact on AP duration (e.g. APD90), amplitude and Vmax (maximum rate of depolarisation) in virtual cardiomyocytes.
- (3) Impedance and field potential measurements were made using human induced pluripotent stem cell cardiomyocytes (iCell2, Cellular Dynamics) on the xCELLigence RTCA CardioECR (ACEA Biosciences) micro-electrode array (MEA) platform. Drugs were exposed for 24h. The following parameters were monitored: Cell Index (CI), Amplitude of contraction, Beat rate, Beating period, Individual Beating Duration (IBD), Field Potential Duration (FPD) and FPD corrected by Fridericia (FPDc), Spike amplitude, Beating Rhythm Irregularity (BRI).

RESULTS

1. Ion channel panel by automated patch-clamp



Patchliner (Nanion Technologies)

	Dilazep	Dipyridamole
	IC_{50} (mM)*	IC_{50} (mM)
hERG	0.9	11.6
hNaV1.5peak	10.6	NE
hNaV1.5late	19.5	NE
hCaV1.2	4.5	NE
hKvLQT1 (Iks)	NE**	NE
hKv4.3 (Ito)	7.7	NE
hKir2.1	NE	NE

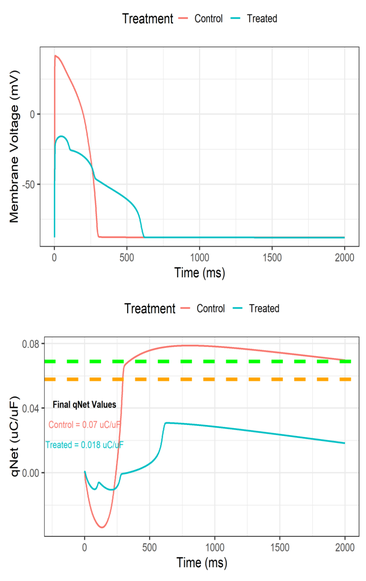
*A Hill Coefficient of 1 was assumed throughout, ** NE No Effect at 30μM

Dilazep demonstrated mixed ion channel block at 5 of the 7 CiPA ion channels. Dipyridamole was active only at hERG

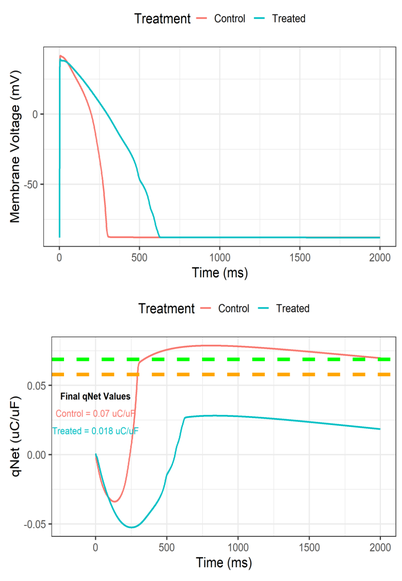
2. AP simulation – *in silico* modelling

1. Input ion channel data
2. Run simulation
3. AP simulation and qNet values were generated

Dilazep, 30μM



Dipyridamole, 30μM

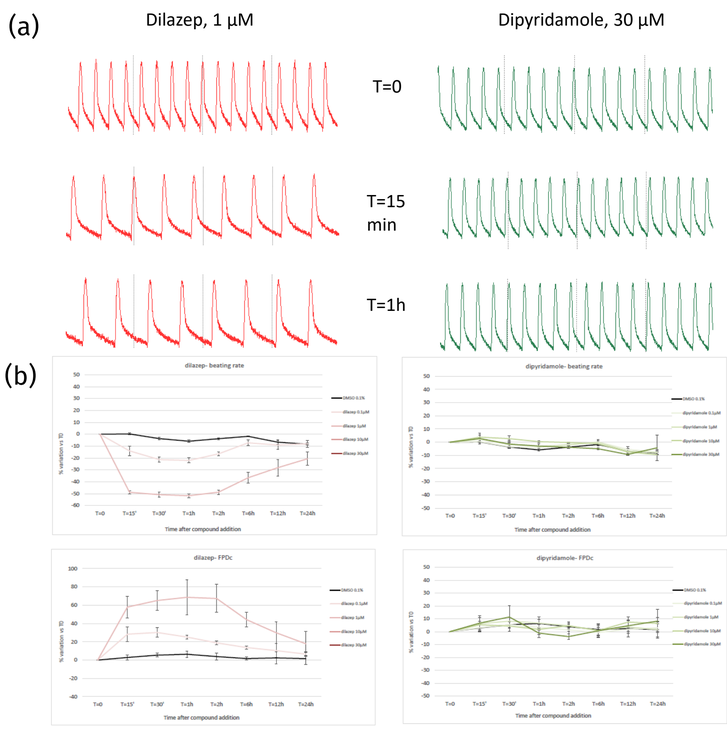


At 30μM, both compounds were predicted to cause a prolongation of action potential duration.

Both compounds qNet value was above the threshold for "high proarrhythmic risk".

3. AP measurement – hiPSC-CMs and MEA

(a) Impedance and (b) Field Potential were measured simultaneously in 48 wells



hiPSC-CM summary	Dilazep	Dipyridamole
Amplitude	NE	NE
Beating rate	↓	NE
Beating period	↑	NE
IBD50	↑	NE
Spike amplitude	↓	NE
Firing rate	↑	NE
FPDc	↑	NE

At 10 and 30μM, dilazep stopped the hiPSC-CMs from beating.

CONCLUSIONS

- By deploying ion channel profiling, *in silico* modelling and field potential measurements *in vitro*, compounds can be classified with different degrees of proarrhythmic risk (low, medium or high).
- At the high concentrations tested, dilazep demonstrated block of multiple ion channels and was predicted to prolong AP duration. This was confirmed in hiPSC-Cardiomyocytes. Therapeutically, this data cannot be put into context because free Cmax concentrations are not available.
- Dipyridamole's only ion channel activity was inhibition of hERG which predicted prolongation of AP duration *in silico*. This finding was not confirmed in hiPSC-CMs. Furthermore, therapeutic free Cmax concentrations of dipyridamole are estimated to be ~30nM (ref. 3). At this concentration, dipyridamole is "low proarrhythmic risk".

1. Noji et al. (2004) Adenosine uptake inhibitors. *Eur. J. Pharmacol.* 495: 1-16
2. CIPAPROJECT.ORG
3. Shultz & Schmoldt (2003) Therapeutic and toxic blood concentrations of more than 800 drugs and other xenobiotics. *Pharmazie* 58: 447-477